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Oriented nanostructures within the human brain uncovered by scanning small angle X-ray scattering

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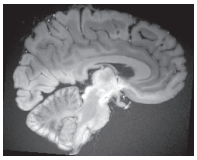
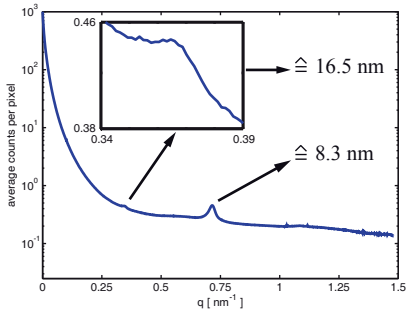
INTRODUCTION

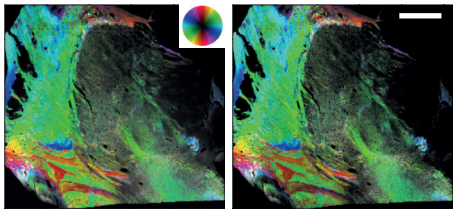


The human brain is one of the most fascinating structures in nature. Histology is so far the gold standard for the differentiation of the different structures on the sub-cellular level. Small-angle X-ray scattering (SAXS) is a reciprocal space technique with an inverse relationship between the size of the inspected particles and scattering angle. Nanostructures within the human brain (e.g. myelin with a periodicity of 16.46 nm) can be detected [1], but it is impossible to relate the results to established histology because of the lack of localization. The combination of SAXS with a spatial resolution of a few micrometers in real space (scanning SAXS at cSAXS beamline, SLS, PSI, Switzerland [2]) provides information on the abundance and orientation of the nanostructures present [3]. The result is a more detailed understanding of the nanoanatomy of human brain tissue.

NO STAIN



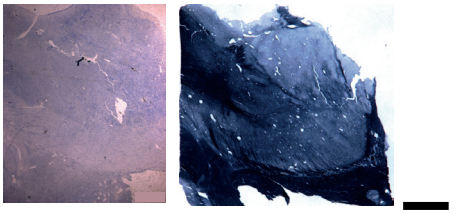
The radial intensity profile exhibits two peaks. The first peak corresponds to a typical myelin periodicity of 16.5 nm. The second peak corresponding to 8.3 nm is the second diffraction order signal.



The figure above shows the signal from an unstained cryosectioned slice, placed in a polyimide sachett, in the range of 8 to 9 nm. In the right image only the signal of the second myelin peak is shown. The bar corresponds to 5 mm. The orientation of the nanostructure is according to the color wheel.

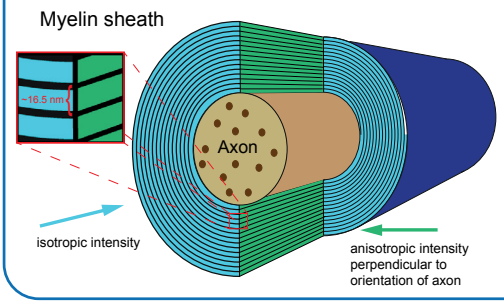
HISTOLOGY

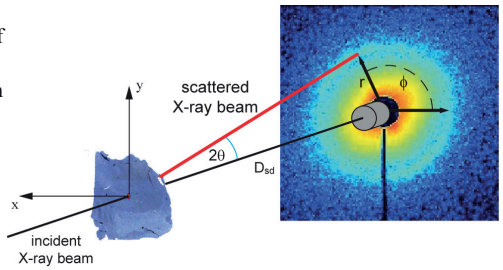
For histologcal analysis, the thalamus block was cryosectioned and stained using NISSL and myelin staining. The images show micrographs of a NISSL (left) and a myelin stained slice (right) used for the scanning SAXS measurements. The length scale bar corresponds to 5 mm.



SCANNING SAXS

- cSAXS beamline (SLS, PSI) using photon energy of 11.2 keV for the section placed in polyimide sachet
- 18.58 keV for histological slices mounted on conventional microscopy glass plates

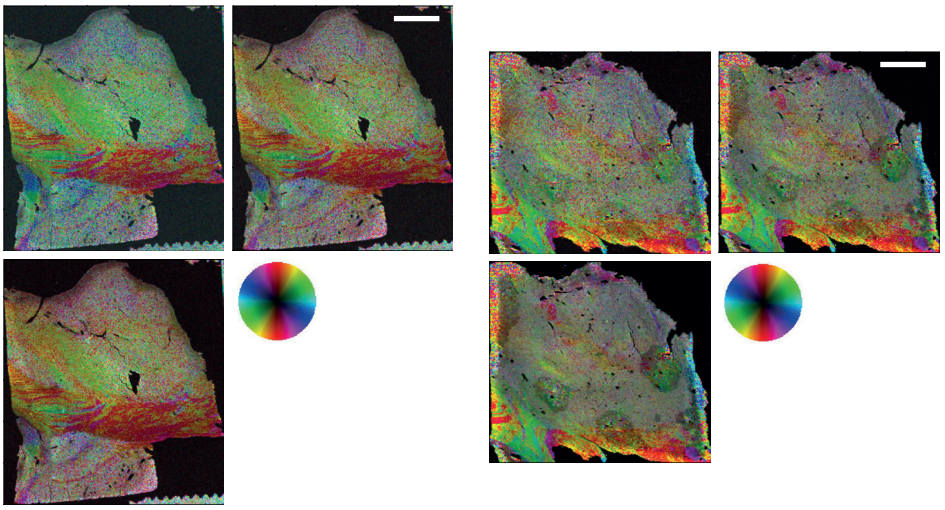




- X-ray beam focused to 20 × 5 µm² and scanned over the slice in 50 and 100 µm steps in the two directions perpendicular to the brain slice
- acquisition rate of 5 images per second - scattering signal recorded using a PILATUS 2M detector placed in a distance of about 7.1 m (D_{sd})

MYELIN & NISSL STAIN

The figure on the left shows the processed scattering signals of a myelin stained thalamus slice mounted on a microscopy glass plate, in the ranges 70 to 80 nm, 102 to 110 nm and 131 to 145 nm from top left to bottom left. The orientation of the scattering signal is according to the color wheel, their brightness is associated with nanostructure abundance. The first image shows no significant orientation inside the thalamus, but in the adjacent region. In the next two ranges, well defined regions with orientations of nanostructures (green, pink) appear inside the thalamus. The figure on the right shows orientation of nanostructures in the ranges of 40 to 50 nm, 50 to 60 nm and 60 to 70 nm (from top left to bottom right) of a NISSL stained thalamus slice mounted on a microscopy glass plate. Here as well, only a few smaller regions inside the thalamus show well defined orientation. The region outside the thalamus, which can be identified with the *internal capsule*, contains strongly oriented nanostructures. The bars correspond to 5 mm.



CONCLUSION AND ACKNOWLEDGEMENT

Scanning SAXS is a powerful imaging technique which allows uncovering the nanostructure of human brain tissue. The method bridges the gap between the real space optical techniques with micrometer resolution and large field of view and the reciprocal space scattering / diffraction techniques with nanometer resolution for average structures, but without imaging capabilities, i.e., spatial resolution across the specimen. In the future scanning SAXS and SAXS tomography will play a dominant role in the further development of nanomedicine and related fields. The valuable contribution of M. Imholz, University Basel and A. Morel, University Hospital Zurich, especially during specimen preparation, are gratefully acknowledged. The project was partially funded by Swiss National Science Foundation (CR23I2_125406).

[1] M. De Felici, et al. (2008) *Physics in Medicine and Biology* ***53****:5675-5688.*
[2] O. Bunk, et al. (2009) *New Journal of Physics* ***11****:123016.*
[3] B. Müller, et al. (2010) *European Journal of Nanomedicine* ***3****:30-33.*